

## FEATURES OF EPITHELIAL-MESENCHYMAL TRANSITION IN ECTOPIC ENDOMETRIUM IN PATIENTS WITH INTRAEPITHELIAL NEOPLASIA OF THE CERVIX

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**Abstract:** Endometriosis is a benign yet invasive disease marked by the ectopic presence of endometrial tissue, significantly impacting women of reproductive age. This study explores the role of epithelial-mesenchymal transition (EMT) in extragenital endometriosis by analyzing the expression of key markers, E-cadherin and vimentin, in various localizations of the disease. Conducted in Moldova and Romania between 2012 and 2017, the study involved immunohistochemical analysis of postoperative specimens from patients aged 19 to 56 years. Results revealed low membrane expression of E-cadherin and pronounced cytoplasmic expression of vimentin in invasive forms, especially in cesarean section scars and inguinal hernias. A contrasting expression pattern was noted in intestinal forms, indicating variations in invasiveness and marker expression based on localization. These findings underscore the critical involvement of EMT in the pathogenesis and invasiveness of extragenital endometriosis, particularly in diffuse-infiltrative forms.

**Keywords:** endometriosis, epithelial-mesenchymal transition, E-cadherin, vimentin, extragenital endometriosis, cesarean section scars, immunohistochemistry, invasiveness.

### Introduction

Endometriosis is a benign disease characterized by the presence of endometrial glands and stroma outside the uterine cavity.

Endometriosis affects 6–10% (about 190 million) of women of reproductive age. The average age of women diagnosed with endometriosis is 28 years.

Although various theories have been proposed to explain the development of endometriosis, its origin remains unclear.

Epithelial-mesenchymal transition (EMT) is a biological process of phenotypic change of an epithelial cell with its transformation into a mesenchymal cell. This phenomenon was first described by Elizabeth Hay [4], who called it "epithelial-mesenchymal transformation" and suggested that in the early stages of embryonic development, epithelial cells can "transform" into cells with a mesenchymal phenotype. In the subsequent period of time, the term "epithelial-mesenchymal transformation" was replaced by the term EMT, which implies the reversibility of the process and does not lead to confusion with tumor transformation. In the embryo, due to EMT, cells with an epithelial phenotype acquire motor activity and colonize new territories.

It is known that epithelial cells are tightly adjacent to each other due to the formation of specialized intercellular connections: nexuses, desmosomes, adhesive and locking contacts. Due to strong connections,

epithelial tissue consists of tightly assembled immobile cells located on the basement membrane separating the epithelium from the connective tissue. The latter is formed by cells of the mesenchymal phenotype, devoid of specialized connections, torn from each other and capable of moving through the intercellular matrix by which they are surrounded.

Membrane proteins, occludins and claudins, participate in the formation of these compounds. Cadherins, transmembrane proteins that provide calcium-dependent intercellular adhesion, play an important role in fixing adjacent zones.

Mesenchymal embryonic cells and fibroblasts from the postnatal period are spindle-shaped cells with cytoplasmic processes (pseudopodia and filopodia), with anterior-posterior polarity ("head-tail"), capable of moving through the intercellular matrix. These cells do not form the mentioned types of intercellular junctions specific to epithelial cells and differ from them by another type of intermediate filaments of the cytoskeleton formed by the protein vimentin. In addition, mesenchymal cells contain another type of transmembrane protein on their surface - N-cadherin.

### Three different types of EMF

The biological significance of EMF is varied, therefore it has been proposed to divide this phenomenon into three different types; the classification was adopted following discussions held within the framework of symposia devoted to EMF in Poland (2007) and the USA (2008) [14, 24].

The first type of EMT is characteristic of embryo implantation and development, formation of organs and tissues, and is manifested in the formation of cells with a common mesenchymal phenotype that do not cause fibrosis and do not spread over long distances by intravasation. Type 2 EMT is associated with the regeneration of mature tissues and organ fibrosis in chronic inflammation. Type 3 EMT is found in neoplastic epithelial cells in invasive carcinoma at the site of the primary tumor; in this case, epithelial cells acquire mesenchymal phenotypic properties and become capable of invasion, intravasation, and metastasis [25, 26].

The invasive aspect demonstrated by microscopic examination of endometriosis is due to changes in the epithelial phenotype under the influence of EMF [27, 28], in which epithelial cells lose intercellular connections, change the cytoskeleton and become mobile. The process of invasion is facilitated by metalloproteinases that affect collagen and have the ability to induce EMF [7, 29]. Thus, the significance of EMF in extragenital endometriosis remains controversial.

### Materials and methods

The study was conducted from 2012 to 2017 in municipal clinics of Chisinau (Republic of Moldova) and Craiova (Romania), in the gynecological and surgical departments. The study included patients aged 19 to 56 years (the average age of patients was  $39.7 \pm 9.9$  years; Md – 38.0, IIQ 32.0–47.0 years) with extragenital endometriosis of various localizations.

The patients lived in the Republic of Moldova ( $n=33$ ; 76.7%; 95% confidence interval (CI) 62.7–87.4) and Romania ( $n=10$ ; 23.3%; 95% CI 12.6–37.3). A minority of patients lived in rural areas (30.2%; 95% CI 18.1–44.9), while the majority were urban residents (69.8%; 95% CI 55.1–81.9).

The most common localizations of extragenital endometriosis were the scar after cesarean section - CS (20.9%; 95% CI 10.9-34.7), appendix (14.0%; 95% CI 6.0-26.5), inguinal hernia (9.3%; 95% CI 3.2-20.6), cecum (7.0%; 95% CI 2.0-17.5), diaphragm (7.0%; 95% CI 2.0-17), jejunum - 3 (7.0%; 95% CI 2.0-17.5), umbilical region (7.0%; 95% CI 2.0-17.5), rectum (7.0%; 95% CI 2.0-17.5), peritoneum (4.7%; 95% CI 1.0–14.1). Other localizations (ascending colon, descending colon, sigmoid colon, transverse colon, ileum, anterior abdominal wall, and retroperitoneal space) were detected in 2.3% (95% CI 0.3–10.4); Fig. 2.

To establish the diagnosis, paraffin blocks corresponding to the postoperative material were used. Using a microtome, 3–5  $\mu\text{m}$  thick sections were obtained from the blocks, which were then stained in a standard manner (hematoxylin and eosin). The stained sections were examined by two pathologists to establish and confirm the diagnosis of endometriosis.

To study the expression of EMT proteins, the immunohistochemical (IHC) method was used. IHC reactions were carried out by the standard method according to the manufacturer's recommendations using 4  $\mu\text{m}$  thick sections on MACH 4 MICRO-POLYMER-HRP devices (Biocare Medical; M4U534). At stage I, the expression of the E-cadherin marker (clone NCH-38; Dako) was assessed, and at stage II, the punctate cytoplasmic expression of vimentin (clone PA1-16759; ThermoFisher) was assessed. Staining of cell parts in brown (when using DAB buffer) and red (when using Vulcan Fast Red buffer), respectively, was considered positive. To assess the expression results, a semi-quantitative method was used with an assessment in points on the Allred scale according to the formula: Total score (TS) = proportion index (PS) + intensity index (IS), TS = 0–8. The proportion was assessed as follows:

1) assessment of the proportion of cells with stained nuclei: 0 – absent; 1 point –  $>0$  to  $1/100$ ; 2 points –  $>1/100$ – $1/10$ ; 3 points – from  $>1/10$  to  $1/3$ ; 4 points – from  $1/3$  to  $2/3$ ; 5 points – from  $2/3$  to 1);

2) intensity score (IS): 0 – no expression; 1 point – weak staining; 2 points – moderate staining; 3 points – strong staining. IHC expression in the cytoplasm (C), cell membrane (Mc) and nucleus (N) was assessed depending on the intensity: C+/Mc+/N+ (weak expression); C++/Mc++/N++ (moderate expression); C++/Mc+++/ N+++ (intense expression). For statistical analysis, we used the  $\chi^2$  concordance criterion, p values  $<0.05$  were considered significant.

## Results

Semi-quantitative analysis of those with a positive reaction to E-cadherin showed a predominance of the result in the two cases studied, while cases demonstrating negative expression of this marker were not taken into account.

Since expression was present at both membranous and cytoplasmic levels, we studied each case of endometriosis separately and tried to correlate these values with some morphological parameters.

According to the obtained results, we noticed a decrease in the membrane expression of E-cadherin in endometriosis after CS, while cytoplasmic expression remained pronounced. Thus, membrane expression was highest in intestinal forms of endometriosis (Fig. 3, a). Positive cytoplasmic expression of vimentin was present in all studied samples, but was especially pronounced in cases of endometriosis in inguinal hernia (Fig. 3, b).

In this study, we did not find a significant difference in the expression of mesenchymal markers in patients with endometriosis. According to the analysis, a more pronounced expression of the E-cadherin marker was recorded in endometriosis of the gastrointestinal tract and a pronounced expression of the vimentin marker was recorded in endometriosis of the anterior abdominal wall after CS, as well as in inguinal hernia.

Positive expression of the E-cadherin marker was detected at both the membrane and cytoplasmic levels in 85.6% of endometriosis cases.

Statistically significant expression of E-cadherin and vimentin correlated as follows: E-cadherin expression was more pronounced, and vimentin expression was less pronounced in intestinal forms of endometriosis. In cases of endometriosis of scars after CS and inguinal hernia, the level of E-cadherin expression was less pronounced, while vimentin expression was predominant, demonstrating a high potential for invasiveness.

## Conclusion

In the vast majority of endometriosis cases, especially in diffuse-infiltrative forms, which occur in particular after CS, the phenotype of lesions is characterized by low expression of E-cadherin, while vimentin expression is at a high level. Such a phenotype proves the involvement of EMT in the pathological process. For comparison, there are differences in the expressivity of markers between superficial and deep lesions, with deep lesions being much more invasive, which is explained by the expression of the corresponding IHC markers.

The results of our study confirmed the role of EMP in the pathogenesis of extragenital endometriosis and prove its invasive potential in these localizations.

## Literature

1. Sh, O. F., Ikhtiyarova, G. A., Xudoyqulova, F. S., & Abdieva, N. U. (2023). EFFECTIVE AND EXPRESS METHOD FOR DIAGNOSING THE CERVICAL AND VAGINA DISEASES IN REPRODUCTIVE AGE WOMEN.
2. Abdieva, N. (2024). CONDITION OF BREAST TISSUE AND THE RISK OF DEVELOPING BREAST CANCER IN PATHOLOGICAL SECRETION SYNDROME. EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE, 4(4), 161-170
3. Abdieva, N. (2024). THE ROLE OF GENETIC PREDICTORS OF METABOLIC DISORDERS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME CONCOMITANT WITH CERVICAL NEOPLASIA. EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE, 4(3), 50-54.
4. Abdiyeva Nigina Ulugbekovna. (2024). ENDOSCOPIC-MORPHOLOGICAL CHARACTERISTICS OF BACKGROUND DISEASES OF THE CERVIX. Лучшие интеллектуальные исследования, 14(4), 120–129.
5. Abdiyeva Nigina Ulugbekovna. (2024). ENDOSCOPIC-MORPHOLOGICAL CHARACTERISTICS OF BACKGROUND DISEASES OF THE CERVIX. Лучшие интеллектуальные исследования, 14(4), 120–129. Retrieved from <http://web-journal.ru/index.php/journal/article/view/3052>.
6. Abdieva, N. U. (2024). THE ROLE OF CYTOKINES IN THE DEVELOPMENT OF CERVICAL ECTOPIA AND ITS PREVENTION. Valeology: International Journal of Medical Anthropology and Bioethics (2995-4924), 2(9), 112-119.
7. Бакиева, М. Ш., Рустамова, Ш. Р., Рахмонов, Т. О., Шарипова, Н. Н., & Мухитдинова, Х. С. (2022). Гипотензивное действие алкалоида бензоилгетератизина на функциональную активность гладкомышечных клеток аорты крысы. AcademicResearchJournalImpactFactor, 7.
8. Samixovna, M. K. (2024). MORPHOLOGICAL DATA OF THE ORGANS OF HEMATOPOIESIS AND HEMATOPOIESIS. Лучшие интеллектуальные исследования, 14(5), 66-74.
9. Samixovna, M. K. (2024). Morphologic Changes in Red Blood Cells. ResearchJournalofTraumaandDisabilityStudies, 3(3), 178-186.
10. Samixovna, M. K. (2024). MORPHOLOGICAL FEATURES OF POSTPARTUM CHANGES IN UTERINE MEMBRANES. SCIENTIFIC JOURNAL OF APPLIED AND MEDICAL SCIENCES, 3(4), 277-283.
11. Samixovna, M. K. (2024). Current Data on Morphological and Functional Characteristics of the Thyroid Gland in Age Groups. JournalofScienceinMedicineandLife, 2(5), 77-83.

12. Samixovna, M. X. (2024). AYOL ORGANIZMI REPRODUKTIV ORGANLARINING RIVOJLANISH XUSUSIYATLARI. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 113-121.
13. Samixovna, M. X. (2024). OITS KASALLIGI, TA'RIFI VA KASALLIKNING KELIB SHIQISH SAVABLARI. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 122-133.
14. Мухиддинова, Х. С. (2024). РАЗВИТИЕ ЯИЧНИКОВ, ИХ МОРФОЛОГИЯ И ОСОБЕННОСТИ ФУНКЦИОНИРОВАНИЕ. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 134-141.
15. Мухитдинова, Х. С. (2024). СОВРЕМЕННЫЕ ВЗГЛЯДЫ НА РАЗВИТИЕ БАКТЕРИАЛЬНОГО ВАГИНОЗА У ЖЕНЩИН ФЕРТИЛЬНОГО ВОЗРАСТА. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 97-103.
16. Мухитдинова, Х. С. (2024). ЗАБОЛЕВАЕМОСТЬ СПИДОМ, МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ БОЛЕЗНИ. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 104-112.
17. Samikhovna, M. K. (2024). Clinical and Morphological Aspects of the Functioning of the Lymphatic System. International Journal of Alternative and Contemporary Therapy, 2(9), 101-106.
18. Samikhovna, M. K. (2024). MODERN VIEWS ON ACROMEGALY AND IMMUNOMORPHOLOGY OF THIS DISEASE. EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE, 4(10), 179-183.
19. Халимова, Ю. С., & Хафизова, М. Н. (2024). МОРФО-ФУНКЦИОНАЛЬНЫЕ И КЛИНИЧЕСКИЕ АСПЕКТЫ СТРОЕНИЯ И РАЗВИТИЯ ЯИЧНИКОВ (ОБЗОР ЛИТЕРАТУРЫ). TADQIQOTLAR. UZ, 40(5), 188-198.
20. Халимова, Ю. С. (2024). Морфологические Особенности Поражения Печени У Пациентов С Синдромом Мэллори-Вейса. Journal of Science in Medicine and Life, 2(6), 166-172.
21. Xalimova, Y. S. (2024). Morphology of the Testes in the Detection of Infertility. Journal of Science in Medicine and Life, 2(6), 83-88.
22. Халимова, Ю. С., & Хафизова, М. Н. (2024). ОСОБЕННОСТИ СОЗРЕВАНИЕ И ФУНКЦИОНИРОВАНИЕ ЯИЧНИКОВ. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 188-194.
23. Хафизова, М. Н., & Халимова, Ю. С. (2024). МОТИВАЦИОННЫЕ МЕТОДЫ ПРИ ОБУЧЕНИИ ЛАТЫНИ И МЕДИЦИНСКОЙ ТЕРМИНОЛОГИИ. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 165-171.
24. Хафизова, М. Н., & Халимова, Ю. С. (2024). ИСПОЛЬЗОВАНИЕ ЧАСТОТНЫХ ОТРЕЗКОВ В НАИМЕНОВАНИЯХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ В ФАРМАЦЕВТИКЕ. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 172-178.
25. Saloxiddinovna, X. Y., & Ne'matillaevna, X. M. (2024). FEATURES OF THE STRUCTURE OF THE REPRODUCTIVE ORGANS OF THE FEMALE BODY. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 55(2), 179-183.
26. Халимова, Ю. С., & Хафизова, М. Н. (2024). КЛИНИЧЕСКИЕ АСПЕКТЫ ЛИЦ ЗЛОУПОТРЕБЛЯЮЩЕЕСЯ ЭНЕРГЕТИЧЕСКИМИ НАПИТКАМИ. TADQIQOTLAR. UZ, 40(5), 199-207.

27. Халимова, Ю. С., & Хафизова, М. Н. (2024). КЛИНИЧЕСКИЕ ОСОБЕННОСТИ ЗАБОЛЕВАНИЙ ВНУТРЕННИХ ОРГАНОВ У ЛИЦ, СТРАДАЮЩИХ АЛКОГОЛЬНОЙ ЗАВИСИМОСТЬЮ. TADQIQOTLAR. UZ, 40(5), 240-250.
28. Халимова, Ю. С., & Хафизова, М. Н. (2024). кафедра Клинических наук Азиатский международный университет Бухара, Узбекистан. Modern education and development, 10(1), 60-75.
29. Abdurashitovich, Z. F. (2024). ANATOMICAL COMPLEXITIES OF JOINT BONES OF THE HAND. EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE, 4(4), 198-206.
30. Зикриллаев, Ф. А. (2024). АНАТОМИЧЕСКОЕ СТРОЕНИЕ ОРГАНОВ ДЫХАНИЯ И ЕГО ЛИЧНЫЕ ХАРАКТЕРИСТИКИ. TADQIQOTLAR. UZ, 40(3), 86-93.
31. Abdurashitovich, Z. F., & Komoliddinovich, S. J. (2024). DIGESTIVE SYSTEM. ANATOMY OF THE STOMACH. TADQIQOTLAR. UZ, 40(3), 78-85.
32. Abdurashitovich, Z. F. (2024). UMURTQA POG'ONASI BIRLASHUVLARI. TADQIQOTLAR. UZ, 40(3), 40-47.
33. Rakhmatova, D. B., & Zikrillaev, F. A. (2022). DETERMINE THE VALUE OF RISK FACTORS FOR MYOCARDIAL INFARCTION. FAN, TA'LIM, MADANIYAT VA INNOVATSIYA JURNALI | JOURNAL OF SCIENCE, EDUCATION, CULTURE AND INNOVATION, 1(4), 23-28.
34. Abdurashitovich, Z. F. (2024). МИОКАРД ИНФАРКТИ UCHUN XAVF OMILLARINING ANAMIYATINI ANIQLASH. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 36(5), 83-89.
35. Abdurashitovich, Z. F. (2024). THE RELATIONSHIP OF STRESS FACTORS AND THYMUS. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 36(6), 188-196.
36. Abdurashitovich, Z. F. (2024). MORPHO-FUNCTIONAL ASPECTS OF THE DEEP VEINS OF THE HUMAN BRAIN. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 36(6), 203-206.
37. Abdurashitovich, Z. F. (2024). ASTRAGAL O'SIMLIGINING TIBBIYOTDAGI MUHIM ANAMIYATLARI VA SOG'LOM TURMUSH TARZIGA TA'SIRI. Лучшие интеллектуальные исследования, 14(4), 111-119.
38. Abdurashitovich, Z. F. (2024). O DAM ANATOMIYASI FANIDAN SINDESMOLOGIYA BO'LIMI HAQIDA UMUMIY MALUMOTLAR. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 41(4), 37-45.
39. Abdurashitovich, Z. F. (2024). THE IMPORTANCE OF THE ASTRAGAL PLANT IN MEDICINE AND ITS EFFECT ON A HEALTHY LIFESTYLE. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 41(4), 88-95.
40. Abdurashitovich, Z. F. (2024). Department of Syndesmology from the Science of Human Anatomy General Information About. Research Journal of Trauma and Disability Studies, 3(3), 158-165.
41. Abdurashitovich, Z. F. (2024). THE COMPLEXITY OF THE FUSION OF THE BONES OF THE FOOT. JOURNAL OF HEALTHCARE AND LIFE-SCIENCE RESEARCH, 3(5), 223-230.
42. Abdurashitovich, Z. F. (2024). MUSHAKLAR TO'GRISIDA MA'LUMOT. MUSHAKLARNING TARAQQIYOTI. MUSHAKLARNING YORDAMCHI APPARATI. TADQIQOTLAR. UZ, 40(3), 94-100.

43. Abdurashitovich, Z. F. (2024). APPLICATION OF MYOCARDIAL CYTOPROTECTORS IN ISCHEMIC HEART DISEASES. ОБРАЗОВАНИЕ НАУКА И ИННОВАЦИОННЫЕ ИДЕИ В МИРЕ, 39(5), 152-159.
44. Abdurashitovich, Z. F. (2024). SIGNIFICANCE OF BIOMARKERS IN METABOLIC SYNDROME. EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE, 4(9), 409-413.